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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 19 July 1999 with an application for Letters Patent number 336814 made by FERNZ CORPORATION LIMITED.

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Dated 9 June 2000.

Neville Harris
Commissioner of Patents

COMMISSIONER OF PATENTS, TRADE MARKS & DESIGNS
NEW ZEALAND

NEW ZEALAND

PATENTS ACT, 1953

PROVISIONAL SPECIFICATION

"Stable Biocidal Compositions"

We, FERNZ CORPORATION LIMITED, a New Zealand company of Manu Street, Otahuhu, Auckland, New Zealand, do hereby declare this invention to be described in the following statement:

The present invention relates to pesticidal compositions, their preparation and their use.

More particularly the present invention relates to multiple active ingredient liquid formulations of a kind where for stability purposes different environments are required for at least two of the active ingredients. For instance, when in an aqueous phase pH requirements for stability differ significantly, eg; a low pH is required for levamisole and a neutral pH is required for abamectin, an ML. Also of the common anthelmintics, levamisole salts are usually much more soluble than morantel salts in aqueous systems, M.L anthelmintics are generally more soluble in organic systems than aqueous systems and benzimidazoles are sparingly soluble.

A particular (but not only) area of interest in stable formulation are those formulations of biocides useful in controlling helminths including nematodes, cestodes, trematodes, and/or ecto-parasites. In this respect the mode of administration including oral, injectable or pour-on (transdermal) routes of such a formulation (whether after aqueous dilution or not) is not critical to the invention.

It is known that simultaneous administration of levamisole and mebendazole [E M Bennet, C Behm, and C Bryant, Int J Parasitology, 1978, 8, 463-466] enhances the anthelmintic activity of benzimidazoles. New Zealand Patent 208288 discloses compositions which contain levamisole and at least one substituted benzimidazole carbamate.

It is also known, based on mathematical modelling that combining 3 or more actives that act to kill a pest in different ways reduces the risk of parasite developing resistance to each of the individual actives if used separately and on their own.

It is also known that it is desirable for ease of use that these actives are combined together in one stable formulation so that administration is by one single administration of an oral, injectable, parenteral or pour-on formulation. It is also desirable that this combined formulation is sufficiently stable so that it can be stored and the formulation used or reused at a later date without degradation of the actives or significant physical changes to the formulations.

Anderson et al in "Mixtures of Anthelmintics: A Strategy Against Resistance" Australian Veterinary Journal, Vol 65, No.2, February 1988, Pages 62-64 suggested that where multiple resistance to broad spectrum nematocides had arisen, treatment with mixtures of nematocides provided effective control of the nematode infections sufficient for use in resistance preventative programs. Hence the need now, much later than 1988, for combined active formulations.

Ancare formulated a double active anthelmintic of levamisole and niclosamide in paraffin oil (LEVITAPE™) to target both tapeworm and roundworm found any water

contamination rendered the product too viscous to use. They tried PEG 6000 wax encasement of niclosamide to protect it from water to no avail. They even tried a detergent based mixture of niclosamide and levamisole but whilst stable it was not safe nor easy to use as a oral drench.

Ancare found that combining benzimidazole drenches with levamisole drenches results in an unstable product due to the different pH values needed to maintain the stability of the individual products. Often, on standing for an hour or two the drenches separate out with the levamisole on top and the benzimidazole left as a sludge on the bottom. It these are not thoroughly mixed again before use the animal may be under or overdosed. A possible consequence of underdosing with the benzimidazole is the build up of a parasite resistant to the benzimidazole.

Merck NZ 183847 discloses avermectin anthelmintic compounds for parenteral administration where they may be carried by a vegetable oil such as peanut oil, cotton seed oil and the like.

Sankyo NZ 199817 refers to oral formulations of two or more anthelmintics where one is a macrolide ("ML") anthelmintic and another may be, for example, a benzimidazole (eg; albendazole), a salicylamide (eg; niclosamide) or an isoquinoline compound (eg; praziquantel). Such formulations are not exemplified by any storage stable formulation although reference is made to formulation as an aqueous solution, as a solution in another suitable non-toxic solvent or as a suspension or dispersion incorporating a suspension aid and a wetting agent (eg; bentonite) or other constituents.

Doramectin is available from Pfizer as DECTOMAX™ as parenteral formulation of sesame oil and ethyl oleate.

Ashmont NZ 280085/280134 discloses injectable ML anthelmintic formulations (eg; abamectin or ivermectin) where a alcohol (such as benzyl alcohol - long since used as a preservative in injectable formulations) acts as a co-solvent with a vegetable oil vehicle (eg; soyabean oil, sesame oil or corn oil). The optional inclusion of ethyl oleate is also disclosed.

Ashmont further discloses (w/w %) a formulation as follows;

ML anthelmintic active	0.5 to 5% w/w
benzyl alcohol	1 to 30% w/w
vegetable oil	to 100% w/w
ethyl oleate	zero or 5 to 30% w/w

Despite combinations above being developed no stable ML anthelmintic and levamisole combination has been marketed. Previous attempts at such a combination resulted in unstable formulation - see our own Examples 1 - 6 hereafter.

The present invention is directed to compositions having at least two actives preferably each usable as an anthelmintic active (albeit with a different spectrum of efficacy with respect to nematodes, trematodes, flukes, etc.) and preferably involving other therapeutic agents.

In another aspect the present invention relates to a benzimidazole containing composition (eg; for pour-on use) where the benzimidazole is solubilised in an organic acid containing phase (eg; lactic acid).

As used herein the term "anthelmintic" and derivatives thereof shall encompass, where the context allows any one or more of a nematocidal, trematocidal and cestocidal active compounds. Where the context so allows "pestocidal" and derivatives thereof shall include any such anthelmintic and any ectoparacidal compound. Where the context allows "ectoparacidal" shall include compounds effective against any one or more of ticks, lice, flies, et al.

In a first aspect the present invention consists in **a storage stable pourable pesticidal composition** having an organic first liquid phase and another ("second") liquid phase, said first phase including at least one active ingredient (and optionally a co-solvent for said active ingredient) and said second phase including a second active ingredient which is substantially insoluble in said first phase **wherein** the presence of an emulsifying agent and/or anti-flocculants ensures stability of the two phases with the first phase as an emulsion within said second phase.

Preferably said second phase is an organic acid and/or aqueous phase.

Preferably said second phase comprises or includes an organic acid such as lactic acid.

Preferably said first phase comprises an oil and/or emollient ester.

Preferably said active ingredients in each of said phases or which might be present as an additional active in both or either phase comprises any of the examples hereinafter provided.

In a second aspect the invention consists in **a stable mix of two or more immiscible liquids or liquid phases** held together by an emulsifier or emulsifiers where one phase is an organic water immiscible phase and another phase is an aqueous or organic phase (eg; water or organic acid(s) such as lactic acid).

In another aspect the present invention consists in **a benzimidazole composition** comprising an organic acid (such as lactic acid) and the benzimidazole dissolved therein.

In yet a further aspect the present invention consists in **a topical or pour-on benzimidazole composition** where the benzimidazole is solubilised in an organic acid (such as lactic acid).

In yet a further aspect the present invention consists in **a benzimidazole**

containing composition where at least one phase is that of an organic acid (such as lactic acid) which solubilises at least one benzimidazole active ingredient.

In another aspect the invention consists in **a ready to use liquid composition** of at least a first active in a first liquid phase and at least a second active in a second liquid phase, the phases forming a stable emulsion.

Optionally a third active may be present in one or other or both of said phases.

Optionally such third active may be suspended in at least one phase.

Preferably each of said first and second actives are dissolved (at least in part) in their respective phases.

In another aspect the present invention consists in **a preferably storage stable pourable composition** comprising or including

up to 25% w/v of at least one pesticide (hereafter "first active(s)") soluble in an organic phase,

1 to 60% w/v of an organic phase [such as a oil (vegetable or mineral) and/or emollient esters] (hereafter "first liquid phase") in which said first active(s) is (are) at least substantially soluble,

0 - 5% w/v of a cosolvent for said first active(s),

1 to 15% w/v of an emulsifying agent,

0 to 20% w/v of at least one further pesticidal active (hereafter "second active(s)") not substantially soluble in said first phase,

and,

means providing a second liquid phase,

said composition having at least most of said first active(s) in a first phase and said first phase being emulsified in the second phase which includes said second active(s).

In another aspect the present invention consists in **a preferably storage stable composition** (whether as a concentrate for aqueous dilution or otherwise) comprising or including

up to 25% w/v of at least one pesticide (hereafter "first active(s)") chosen from the class of at least partly oil or emollient ester soluble actives,

1 to 60% w/v of at least one water immiscible organic phase [such as a oil (vegetable or mineral) and/or emollient esters] in which said first active(s) is (are) at least substantially soluble,

0 - 5% w/v of a cosolvent for said first active(s),

1 to 15% w/v of an emulsifying agent,

0 to 20% w/v of at least one further pesticidal active (hereafter "second active(s)") not substantially soluble in said organic phase which is (i) dissolved in said water and/or (ii) suspended in said water,

and,

water,

said composition having an organic phase with at least most of said first active(s), said organic phase being emulsified in an aqueous phase of said water and said second active(s).

Preferably is one embodiment said first and second active are anthelmintics.

Preferably said organic phase at least includes an oil.

Preferably additional active ingredients are included in said composition whether in said organic phase or said aqueous phase or a mixture of them both.

In one preferred form of the present invention an additional active ingredient may be included in such a way that the composition is a suspo-emulsion.

In another aspect the present invention consists in **a storage stable pesticidal composition** having an organic phase and an aqueous phase, said organic phase being of an oil and/or emollient ester which includes at least one active ingredient (and optionally a co-solvent for said active ingredient) and an aqueous phase including a second active ingredient which is substantially insoluble in said organic phase **wherein** the presence of an emulsifying agent and/or anti-flocculants ensures stability of the two phases with the organic phase as an emulsion within said aqueous phase.

Preferably said composition includes still further actives which as particles, liposomes or as liquid globules are suspended in one or other or both of said phases. For instance preferably said first active may be a broad spectrum anthelmintic such as an ML anthelmintic (eg; abamectin) and said second active is a water soluble active capable of killing nematodes such as levamisole. An additional active or additional actives may includes one or more of suitable actives dissolvable or suspendible or emulsifiable in one or other of the phases having, for example, an additional anthelmintic, or an active with an ecto-parasitical effect or a flukicidal effect.

Equally this may contain two or more actives that have activity against trematodes such as Flukes or Cestodes, or Ectoparasites.

In another aspect the present invention consists in **a pourable pesticidal composition** comprising or including

at least one active ingredient chosen from the class of macro cyclic lactones (hereafter "ML"),

at least one active ingredient chosen from the tetramisole/levamisole class,

at least one organic liquid carrier (preferably at least one oil and, optionally, at least one organic co-solvent) to provide a first liquid phase, and

means providing a second liquid phase,

and, optionally, an emulsifying agent or agents,

wherein said ML active ingredient(s) is(are) at least primarily in the first phase in solution,

and wherein said tetramisole/levamisole class active ingredient(s) is(are) at least primarily in solution in the second phase,

and wherein said phases exist in, or can be shaken into, the form of an emulsion.

In another aspect the present invention consists in **a pesticidal composition** (whether as a concentrate for aqueous dilution or otherwise) comprising or including

at least one active ingredient chosen from the class of macro cyclic lactones (hereafter "ML"),

at least one active ingredient chosen from the tetramisole/levamisole class,

at least one organic liquid carrier (preferably at least one oil and, optionally, at least one organic co-solvent), and

water,

and, optionally, an emulsifying agent or agents,

wherein said ML active ingredient(s) is(are) at least primarily in the organic liquid carrier(s) in solution (hereafter referred to as "the organic phase"),

and wherein said tetramisole/levamisole class active ingredient(s) is(are) at least primarily in solution in the water (hereafter referred to as "the aqueous phase"),

and wherein said organic phase and said aqueous phase exist in, or can be shaken into, the form of an emulsion.

Optionally a co-solvent is present. Preferably such co-solvent is selected from the class of alcohols having multiple carbons (preferably 4 or more) (eg; benzyl alcohol), diols and glycol ethers.

Preferably a third active ingredient is included in the composition.

In one form preferably said third ingredient (if an anthelmintic) is other than an ML anthelmintic and other than an anthelmintic chosen from the tetramisole/levamisole class.

Preferably said third anthelmintic agent is selected from the group including benzimidazoles and probenzimidazoles (eg; albendazole, oxfendazole, triclabendazole), praziquantel, salicylamides (eg; closantel), organophosphates, (napthalophos), clorsulony, nitroxynil, morantel, pyrantel, et al. - see Table 2 - Spectrum of Activity.

Preferably said third anthelmintic active ingredient is suspended at least primarily in the aqueous phase.

Preferably said aqueous phase includes at least one suspension agent for said third anthelmintic active ingredient.

In still other aspects the present invention consists in **a (preferably storage stable) pourable composition** comprising or including

at least one anthelmintic active (hereafter "first anthelmintic active(s)"),

at least one liquid to provide a first phase in which said first anthelmintic active(s) is (are) at least substantially soluble,

an emulsifying agent,

at least one liquid to provide a second phase, and

at least one further anthelmintic active (hereafter "second anthelmintic active(s)") not substantially soluble in said first phase,

said composition having a first phase with at least most of said first anthelmintic active(s) and a second phase of at least most of said second anthelmintic active(s).

In still other aspects the present invention consists in **a (preferably storage stable) composition** (whether as a concentrate for aqueous dilution or otherwise) comprising or including

at least one anthelmintic (hereafter "first anthelmintic active(s)") chosen from the class of at least partly oil soluble anthelmintic actives,

at least one oil (optionally also with an organic co-solvent) in which said first anthelmintic active(s) is (are) at least substantially soluble,

an emulsifying agent,

water, and

at least one further anthelmintic active (hereafter "second anthelmintic active(s)") not substantially soluble in said oil(s) which is (i) dissolved in said water and/or (ii) suspended in said water,

said composition having an organic phase of said oil(s) with at least most of said first anthelmintic active(s) and an aqueous phase of said water and at least most of said second anthelmintic active(s).

Preferably said oil is a vegetable oil or a mineral oil.

Preferably said composition includes a third active.

Preferably said third active is a therapeutic agent such as an antimicrobial and/or still further anthelmintic active (eg; flukicide) and/or an ecto-parasite (hereinafter "third active(s)").

Preferably said third active(s) is primarily disposed within said aqueous phase although in other forms of the present invention it may be partitioned between the organic and aqueous phases or largely in the organic phase.

Preferably said second anthelmintic active(s) is dispersed substantially homogeneously as a suspension in the water.

Preferably said composition includes any one or more of the following:

- a co-solvent which is to form part of said aqueous phase, eg; as maybe preferable if the first anthelmintic active(s) is only partially soluble in the vegetable oil(s),

- mineral(s),
- vitamin(s),
- antimicrobial(s),
- anthelmintic(s),
- antifreeze(s),
- thickening agent(s),
- anti-focculant(s),
- pH stabiliser(s).

Preferably said vegetable oil(s) is selected from the group including soyabean oil, sesame seed oil, corn oil, sunflower oil, peanut oil and safflower oil.

Preferably said emulsifying agent is Tween 80 and/or Teric 380.

Preferably said second anthelmintic active is selected from the group consisting of levamisole or tetramisole (if a water phase soluble anthelmintic) or a water phase suspendible or dispersible anthelmintic such as a suitable benzimidazole typified by, for example, albendazole or oxfendazole. Alternatively said optional third active is a dispersed benzimidazole.

Preferably said cosolvent is selected from the group including high chain alcohols (such as benzyl alcohol), diols, glycol ethers, and esters (eg; PMR).

Preferably said antimicrobial is selected from the group including benzoic acid, potassium sorbate and parabens.

Preferably said minerals are selected from the group including mineral salts or chelates, eg; selenium, copper, cobalt and zinc.

Preferably said vitamins are selected from the group including vitamins A, D, E, B and C.

Preferably said antifreeze or freezes is or are selected from the group consisting of propylene glycol and glycerine.

Preferably said thickening agent are selected from the group of cellulose gum, xanthum gum, carbapol and algeinates.

Preferably said anti-flocculants are selected from the group consisting of Terasperse 2500 and 4896 (preferably both).

Preferably said pH stabilisers are selected from the group consisting of citric acid, phosphate salts, etc.

Preferably the or a said organic liquid carrier is an oil.

Preferably said oil is a vegetable oil. In other forms it is a mineral oil (eg; medium grade).

Preferably said vegetable oil is selected from a group consisting of or including soyabean oil, corn oil, sesame seed oil, sunflower oil, peanut oil and safflower oil.

In yet a further aspect the present invention is **a stable formulation** of

a first active in an organic ("first") phase,

a second active in a second liquid phase, and

additional actives in one or other, or both, said phase(s),

and wherein said phases provide a stable emulsion,

and wherein at least one (and preferably both) of said first and second actives is (are) an anthelmintic active.

Preferably a third active is a suspended active at least primarily in the second phase (eg; an anthelmintic or ectoparasiticide).

Preferably an emulsifying agent assists in the stability of the emulsion.

In yet a further aspect the present invention is **a stable formulation** of

a first active in an organic phase at least primarily of oil(s),

a second active in an aqueous phase, and

additional actives in one or other, or both, said organic and aqueous phase(s),

and wherein said organic and aqueous phases provide a stable emulsion,

and wherein at least one (and preferably both) of said first and second actives is (are) an anthelmintic active.

Preferably a third active is a suspended active at least primarily in the aqueous phase (eg; an anthelmintic or ectoparasiticide).

Preferably an emulsifying agent assists in the stability of the emulsion.

In a further aspect the invention consists in **a method of formulating an anthelmintic composition** of a kind having

at least one anthelmintic (hereafter "first active(s)"),

a liquid or liquids to define a first phase in which said first active(s) is (are) at least substantially soluble,

(optionally) an emulsifying agent,

at least one further anthelmintic active (hereafter "second active(s)") not substantially soluble in said first phase

(optionally) anti-flocculant(s),

and,

a liquid or liquids to define a second phase,

said method comprising or including the steps of

(I)(a) providing a mix of said first active ingredient and at least the first phase liquid(s),

(b) providing a mix of said second active ingredient and at least the second phase liquid(s), and

(II) by mixing at least the mixes of (I)(a) and (I)(b) forming an emulsion with

at least most of said first active in the first phase and at least most of the second active in the second phase.

In a further aspect the invention consists in **a method of formulating an anthelmintic composition** of a kind having

at least one anthelmintic (hereafter "first active(s)") chosen from the class of (at least partly) oil soluble anthelmintic actives,

an oil or oils in which said first active(s) is (are) at least substantially soluble,

optionally a cosolvent for said first active(s),

an emulsifying agent,

at least one further anthelmintic active (hereafter "second active(s)") not substantially soluble in said oil(s),

optional anti-flocculant(s),

and,

water,

said method comprising or including the steps of

(I)(a) providing a mix of said first active ingredient, the oil(s), the optional co-solvent(s) and the emulsifying agent(s),

(b) providing a mix of said second active ingredient the water, and the optional anti-flocculant(s), and

(II) by mixing at least the mixes of (I)(a) and (I)(b) forming an emulsion with at least most of said first active in the oil(s) and at least most of the second active in the aqueous phase.

In yet a further aspect the invention consists in **an anthelmintic composition** so made.

In still other aspects the invention is **a method of treating mammals for pests** which involves (whether with dilution or not) administering or having self administered to such mammals effective amounts of active(s) of compositions of the present invention.

In still a further aspect the invention consists in **the use of an anthelmintic composition** of any of the kinds previously defined.

In some forms said use is as an oral, a pour-on or as a parenteral composition (preferably with an anthelminticly effective amount of each active).

Preferred formulations of the present invention comprise:

Component 1 - water insoluble but mostly or completely oil soluble anthelmintic active.

Component 2 - An oil (vegetable or mineral)

Component 3 - an emulsifying agent

Component 4 - water

Component 5A, 5B, etc. - Additional active(s) of which at least one is preferably an anthelmintic active. This or these can be either dissolved in the water phase (such as Levamisole) or suspended in the water phase (such as Albendazole) if insoluble, or both (eg; Levamisole and Albendazole).

The water insoluble active is dissolved in the oil, which is emulsified in water. The oil protects the active against the pH or constituents present in the water phase.

Additional components can be added to this basic formulation.

Addition 1 - if component 1 is only partially soluble in the oil phase then a co-solvent may be necessary (eg; an organic co-solvent).

Addition 2 - Minerals/Vitamins

Addition 3 - An antimicrobial.

Addition 4 - Antifreezes.

Addition 5 - Thickening agents.

Addition 6 - Anti-flocculants.

Addition 7 - pH stabilisers.

Preferred components and their concentration ranges and examples are set out in Table 1.

Table 1	Function	Examples	% W/V
Component 1	Active	Abamectin	0 - 25%
Component 2	Vegetable Oil Mineral Oil	Soya bean Medium Grade	1 - 60% 1 - 60%
Component 3	Emulsifying Agent	Tween 80 Teric 380	1 - 15%
Component 4	Water		To Volume
Component 5A, 5B, etc.	Additional anthelmintic Water soluble Water Insoluble	 Levamisole Albendazole	 0 - 15% 0 - 20%
Addition 1	Co-Solvent	Includes high chain Alcohols (such as Benzyl Alcohol), diols such as PMP (promyristyl propionate), Glycol Ethers and Esters	0 - 5%
Addition 2	Minerals	Mineral salts or chelates	0 - 5%
Addition 3	Antimicrobials	Benzoic Acid, Potassium Sorbate and Parabens	0 - 1%
Addition 4	Antifreeze	Propylene Glycol, Glycerine	0 - 5%
Addition 5	Thickening Agents	Cellulose Gum, Xanthum Gum, Carbapol and Algeinates	0 - 3%
Addition 6	Anti-flocculants	Terasperse 2500 and 4896	0 - 7%
Addition 7	pH Stabilisers	Citric Acid, Phosphate salts etc.	0 -05%

Preferred actives by reference to activity are now tabulated.

Table 2 - Spectrum of Activity

Compound	Nematodes		Cestodes	Trematodes	Protozoa	Ectoparasites			
	Gastrointestinal Worms	Lung-worms	Tapeworms	Fluke		Lice	Mites	Ticks	Flies
A. Macrocyclic Lactones									
1. Avermectins									
Ivermectin	+	+	-	_		+	+	+	+
Abamectin	+	+	-	-		+	+	+	+
Doramectin	+	+	_	_		+	+	+	+
Eprinomectin	+	+				+	+	+	+
2. Milbemycin									
Moxidectin	+	+	-	-		+	+	+	+
B. Benzimidazoles									
1. Probenzimidazoles									
Febantel	+	+							
Netobimin	+	+							
2. Benzimidazoles (thiazoles)									
Thiabendazole	+	+							
Cambendazole	+	+							
3. Benzimidazoles (carbamates)									
Albendazole	+	+	+	+	+(Giardia)	-	-	-	-
Fendendazole	+	+		+	+(Giardia)	-	-	-	-
Mebendazole	+	+				-	-	-	-
Rycobendazole	+	+	+	+		-	-		
Oxfendazole	+	+	+	+		-	-		
Parrbendazole	+	+		-					
Triclabendazole	-	-	-	+		-	-		-
C. Imidazothiazole									

Compound	Nematodes		Cestodes	Trematodes	Protozoa	Ectoparasites			
Levamisole	+	+							
D. Tetrahydropyrimidazole									
Morantel	+		+						
Pyrantel	+								
E. Salicylamidides									
Closantel	+(Haemonchus)	-	-	+					+
Oxyclosanide	+(Haemonchus)			+					
Rafoxamide	+(Haemonchus)								
Niclosamide			+						
F. Benzoenedisuplhonamide									
Clorsulon	-	-	-	+					
G. Nitrophenolic Compounds									
Nitroxyinil	+(Haemonchus)	-	-	+					+
H. Pyrazinoisoquinoline									
Praziquantel	-	-	+						
I. Organophosphates									
Napthalophos	+								
Trichlorphon	+								+
Propetamphos									+
Diazinon						+	+		+
Cumaphos						+	+	+	+
J. Synthetic Pyrethroids									
Cypermethrin						+			+

Compound	Nematodes		Cestodes	Trematodes	Protozoa	Ectoparasites			
Alphamethrin						+			+
Flumethrin								+	
K. Insect Growth Regulators									
Diflubenzuron						+			+
Triflumuron						+			
Cyromazine									+
L. Amitraz								+	
M. Cyhalothrin								+	+
N. GABA Inhibitor									
Fipronil						+	+	+	+

In the following examples all percentages are weight to volume. These examples show various formulations directed to a resistence strategy which nevertheless are stable and may have additional therapeutic and/or active inclusions.

EXAMPLE 1

Combination Abamectin/Levamisole Drench

Abamectin	0.20 % w/v
Tween 80	8.00 % w/v
Benzyl Alcohol	3.00 % w/v
Propylene Glycol	20.00 % w/v
Na_2HPO_4	1.03 % w/v
Citric Acid	0.29 % w/v
Levamisole HCL	8.00 % w/v
Sodium Selenate	0.24 % w/v
Water	To Volume % w/v

This was formulated as follows at ambient temperature.

- Mix 1 - Dissolved Abamectin in Benzyl Alcohol, mixed in Tween and Propylene Glycol.
- Mix 2 - Dissolved Levamisole, Na_2HPO_4, Citric acid and Sodium Selenate in

water.

- Combined mix 1 and 2.

This resultant formulation was physically stable but considered too thin.

EXAMPLE 2

Combination Abamectin/Levamisole Drench

Abamectin	0.20 % w/v
Tween 80	8.00 % w/v
Benzyl Alcohol	3.00 % w/v
Propylene Glycol	20.00 % w/v
Na_2HPO_4	1.03 % w/v
Citric Acid	0.29 % w/v
Levamisole HCL	8.00 % w/v
Sodium Selenate	0.24 % w/v
Cellulose Gum CMC	0.50 % w/v
Water	To Volume % w/v

This was formulated as follows;

- Mix 1 - Dissolved Abamectin in Benzyl Alcohol then add Tween.
- Mix 2 - Dissolved Levamisole, Na_2HPO_4, Citric acid and Sodium Selenate in water.
- Mix 3 - Dispersed thickener in Propylene glycol.
- Combined Mix 2 and 3 then added Mix 1.

EXAMPLE 3

Combination Abamectin/Levamisole Drench

Abamectin	0.20 % w/v
Tween 80	8.00 % w/v
Benzyl Alcohol	3.00 % w/v
Propylene Glycol	20.00 % w/v
Na_2HPO_4	1.03 % w/v
Citric Acid	0.29 % w/v
Levamisole HCL	8.00 % w/v
Sodium Selenate	0.24 % w/v
Water	To Volume % w/v

EXAMPLE 4

Combination Abamectin/Levamisole Drench

Abamectin	0.20 % w/v
Tween 80	8.00 % w/v
Benzyl Alcohol	3.00 % w/v
Propylene Glycol	20.00 % w/v
Na_2HPO_4	1.03 % w/v
Citric Acid	0.29 % w/v
Levamisole HCL	8.00 % w/v
Sodium Selenate	0.24 % w/v
Water	To Volume % w/v

The low pH of these formulations 2, 3 and 4 (pH<4) was identified as unsuitable for long the long-term stability of Abamectin. These completely aqueous formulation approaches were then stopped and it was decided to use a vegetable oil to attempt to encapsulate the Abamectin and possibly protect if from the low pH of the water phase.

EXAMPLE 5

Combination Abamectin/Albendazole/Levamisole Drench

Abamectin	0.10 % w/v
Albendazole	2.50 % w/v
Benzyl Alcohol	3.00 % w/v
Teric 215	10.0 % w/v
Teric 216	10.0 % w/v
Propylene Glycol	3.00 % w/v
Na_2HPO_4	1.05 % w/v
Citric Acid	1.21 % w/v
Levamisole HCL	3.75 % w/v
Sodium Selenate	0.12 % w/v
Cobalt EDTA	0.36 % w/v
Soyabean Oil	3.00 % w/v
Xanthum gum	0.32 % w/v
Water	To Volume % w/v

This was formulated as follows:

- Mix 1 - Dissolve Abamectin in Benzyl Alcohol. Mix in Terics.
- Mix 2 - Dissolve Levamisole, Cobalt-ETDA, Citric acid, Na_2HPO_4 and Sodium

Selenate in water.

- Mix 3 - Disperse Xanthum gum in Propylene glycol then add 5% of water to form gel.
- Combine Mix 1 and 2. Added Albendazole. Then added Mix 1.

This formulation after 2 months accelerated stability assays shows that the Abamectin was degrading. The physical stability of this formulation was also poor with Albendazole flocculating out and with the oil phase showing evidence of curdling.

EXAMPLE 6

Combination Abamectin/Albendazole/Levamisole Drench

Abamectin	0.10 % w/v
Albendazole	------
Benzyl Alcohol	3.00 % w/v
Teric 215	10.0 % w/v
Teric 216	10.0 % w/v
Propylene Glycol	3.00 % w/v
Na_2HPO_4	1.05 % w/v
Citric Acid	1.21 % w/v
Levamisole HCL	3.75 % w/v
Sodium Selenate	0.12 % w/v
Cobalt EDTA	0.36 % w/v
Soyabean Oil	3.00 % w/v
Xanthum gum	0.32 % w/v
Water	To Volume % w/v

This formulation like that of Example 6 after 2 month accelerated stability assays also showed that Abamectin was degrading. The physical stability of this formulation was also poor with Albendazole flocculating out and with the oil phase showing evidence of curdling.

To prevent such flocculation Terasperse 4896 and Terasperse 2500 were then trialed. These theoretically coat the Albendazole and *improve the solubility in water.*

Teric 380, a more appropriate emulsifying agent for stable vegetable oil emulsions.

The percentage of the oil phase was increased to 10% to increase the partition between the oil/water phase possibly improve Abamectin stability.

EXAMPLE 7

Combination Abamectin/Albendazole/Levamisole Drench

Abamectin	0.10 % w/v
Albendazole	1.90 % w/v
Benzyl Alcohol	3.00 % w/v
Terasperse 4896	1.00 % w/v
Terasperse 2500	2.00 % w/v
Teric 380	5.00 % w/v
Propylene Glycol	3.00 % w/v
Levamisole HCl	4.00 % w/v
Sodium Selenate	0.12 % w/v
Cobalt EDTA	1.50 % w/v
Soyabean Oil	10.00 % w/v
Xanthum gum	0.20 % w/v
Water	To Volume % w/v

This was formulated as follows:

- Mix 1 - Dissolved Abamectin in Benzyl Alcohol. Mix into soyabean oil. Mix in Teric 380.
- Mix 2 - Added Albendazole and Terasperses to water. Added Levamisole, Cobalt-ETDA. Citric acid and Sodium Selenate.
- Mix 3 - Dispersed Xanthum gum in Propylene glycol then added 5% of water to form gel.
- Combined Mix 1, 2 and 3.

This formulation gave no flocculation but the oil phase readily separated out.

The oil/water phase required further stabilisation. A series of water/oil/emulsifier blends were prepared to optimise this aspect of the formulation. Tween 80 was also trialed as a possible alternative emulsifier to Teric 380. All formulations were stored at ambient.

EXAMPLE 8

Oil/Water Blends

Tween 80	10.0 % w/v
Soyabean Oil	10.0 % w/v
Water	To Volume % w/v

This was prepared as a simple mixture but the emulsion broke after 4 hours.

EXAMPLE 9

Oil/Water Blends

Teric 380	5.0 % w/v
Soyabean Oil	10.0 % w/v
Water	85.0 % w/v

This straight blend broke after 2 days.

EXAMPLE 10

Oil/Water Blends

Tween 80	10.0 % w/v
Soyabean Oil	50.0 % w/v
Water	40.0 % w/v

This straight blend emulsion broke after 4 days.

EXAMPLE 11

Oil/Water Blends

Teric 380	5.0 % w/v
Soyabean Oil	50.0 % w/v
Water	45.0 % w/v

This straight blend emulsion broke after 25 days.

As a result we concluded that

Teric 380 was the better emulsifier, and

the higher concentration of oil emulsion was the most stable option. Therefore we decided to trial with oil at from 35 to 60% w/v.

EXAMPLE 12

40 % Soyabean Oil

Abamectin	0.10 % w/v
Albendazole	1.90 % w/v
Benzyl Alcohol	3.00 % w/v
Terasperse 4896	1.00 % w/v
Terasperse 2500	2.00 % w/v
Teric 380	5.00 % w/v
Propylene Glycol	3.00 % w/v
Levamisole HCl	4.00 % w/v
Sodium Selenate	0.12 % w/v
Cobalt EDTA	1.50 % w/v
Soyabean Oil	40.00 % w/v
Xanthum gum	0.30 % w/v
Water	To Volume % w/v

This was formulated as follows at ambient temperature.

- Mix 1 - Dissolved Abamectin in Benzyl Alcohol. Mix into Soyabean oil. Mix in Teric 380.
- Mix 2 - Added albendazole and Terasperses to water. Added levamisole, Cobalt-ETDA, Citric acid and Sodium Selenate.
- Mix 3 - Dispersed Xanthum gum in Propylene glycol then added 5% of water to form gel. Combined Mix 1, 2 and 3.

With this formulation we found no flocculation occurred or separation of the oil was observed. This batch has remained stable after being recycling between 4°C, ambient and 30°C for 6 months.

Although formulation of Example 12 was showing indications of suitability another formulation option was tested. Xanthum Gum was replaced with Alginate as a alternative thickener.

EXAMPLE 13

Combination Abamectin/Albendazole/Levamisole Drench with alginates

Abamectin	0.10 % w/v
Albendazole	1.90 % w/v
Benzyl Alcohol	3.00 % w/v
Terasperse 4896	1.00 % w/v

Terasperse 2500	2.00 % w/v
Teric 380	5.00 % w/v
Propylene Glycol	3.00 % w/v
Levamisole HCL	4.00 % w/v
Sodium Selenate	0.12 % w/v
Cobalt EDTA	1.50 % w/v
Soyabean Oil	10.00 % w/v
Alginate (Kelcolid)	0.20 % w/v
Water	To Volume % w/v

Formulating Order:

- Mix 1 - Dissolved Abamectin in Benzyl Alcohol. Mix into soyabean oil. Mix in Teric 380.
- Mix 2 - Added Albendazole and Terasperses to water. Added Levamisole, Cobalt-ETDA, Citric acid and Sodium Selenate.
- Mix 3 - Dispersed Alginate in Propylene glycol then added 5% of water to form gel.
- Combined Mix 1, 2 and 3.

Findings:

- Product appeared stable but a rapid viscosity drop with time was observed. This was followed by eventual slight separation of the water phase in long term stability samples.

EXAMPLE 14 (Batch No. LB99/01)

Increasing Active Loading

Abamectin	0.10 % w/v
Albendazole	**2.38 % w/v**
Benzyl Alcohol	3.00 % w/v
Terasperse 4896	1.00 % w/v
Terasperse 2500	2.00 % w/v
Teric 380	5.00 % w/v
Propylene Glycol	3.00 % w/v
Levamisole HCL	3.75 % w/v
Soyabean Oil	40.00 % w/v
Xanthum gum	0.30 % w/v
Water	To Volume % w/v

Formulating Order:

- Mix 1 - Dissolved Abamectin in Benzyl Alcohol. Mix into soyabean oil. Mix in Teric 380.
- Mix 2 - Added Albendazole and Terasperses to water. Added Levamisole and Citric Acid.
- Mix 3 - Dispersed Xanthum gum in Propylene glycol then added 5% of water to form gel.
- Combined Mix 1, 2 and 3.

Findings:

- Product stable under an accelerated stability programme.

The robustness of this formulation concept was examined by substituting Albendazole with Oxfendazole and increasing active loading.

EXAMPLE 15 (Batch No. LB99/03)
Substituting Albendazole with Oxfendazole

Abamectin	0.10 % w/v
Oxfendazole	2.26 % w/v
Benzyl Alcohol	3.00 % w/v
Terasperse 4896	1.00 % w/v
Terasperse 2500	2.00 % w/v
Teric 380	5.00 % w/v
Propylene Glycol	3.00 % w/v
Levamisole HCL	3.75 % w/v
Soyabean Oil	40.00 % w/v
Xanthum gum	0.30 % w/v
Water	To Volume % w/v

Formulated similarly to Example 14.

Findings:

- Product stable under an accelerated stability programme.

EXAMPLE 16

Increasing Active Loading

Abamectin	0.10 % w/v
Albendazole	5.00 % w/v
Benzyl Alcohol	3.00 % w/v
Terasperse 4896	2.00 % w/v
Terasperse 2500	4.00 % w/v
Teric 380	5.00 % w/v
Propylene Glycol	3.00 % w/v
Levamisole HCL	3.75 % w/v
Soyabean Oil	35.00 % w/v
Xanthum gum	0.30 % w/v
Water	To Volume % w/v

Prepared similarly to Example 14.

Findings:

- Product stable under an accelerated stability programme.

EXAMPLE 17

Benzimidazole Active in Lactic Acid

Carnola Oil	40.00 %
Teric 380	5.00 %
Abamectin	0.10%
Promyristyl Propionate	3.00 %
Triclabendazole	-------
Oxfendazole	4.00 %
Levamisole HC1	-------
Lactic Acid	50.0%

Formulated Order:

- Mix 1 - Dissolve Abamectin in warmed emollient Ester (Promyristyl Propionate) then mix into Oil. Mix in Teric 380.
- Mix 2 - To Lactic acid dissolve Oxfendazole or Levamisole and Triclabendazole (slight heat required).
- Mix 3 - Combine mixes 1 and 2 with high shear agitation

Findings:

- Suitable as a pour-on formulation. May include a thickener.

EXAMPLE 18

Benzimidazole Active in Lactic Acid

Carnola Oil	40.00 %
Teric 380	5.00 %
Abamectin	0.10%
Promyristyl Propionate	3.00 %
Triclabendazole	0.5%
Oxfendazole	-------
Levamisole HC1	3.75%
Lactic Acid	50.0%

Formulated Order:

- Mix 1 - Dissolve Abamectin in warmed emollient Ester (Promyristyl Propionate) then mix into Oil. Mix in Teric 380.
- Mix 2 - To Lactic acid dissolve Oxfendazole or Levamisole and Triclabendazole (slight heat required).
- Mix 3 - Combine mixes 1 and 2 with high shear agitation

Findings:

- Suitable as a pour-on formulation. May include a thickener.

TRIALS

- **Cattle**

Thirteen mixed breed weaner beef cattle (90-153 kg) naturally infected with worms were divided into four treatment groups by weight and nematode faecal egg count (FEC) into three groups of three animals and a fourth group of four animals.

Animals in group 2 were treated with the test combination formulation orally (Batch LB99/03, see example 15) at a dose of 1mL per 5 kg bodyweight. Group 3 of four animals had the same product (Batch LB99/03) applied as a pour-on along the midline of the upper back at a dose of 1 mL per 2.5 kg bodyweight. Group 4 received the same dose rate of the three actives as in group 2 given orally using three separate commercial formulations. Group 1 received no treatment (untreated control animals).

Animals were treated at Day 0 of the trial. Faeces for faecal egg count and blood samples for blood levels of active were collected at various times following treatment.

Slaughter was at Day 14 of the trial where the total number of worms were counted in the gastrointestinal tract.

The results are presented below and in the drawings.

Table 3

Treatment Groups

Group	No. of Animals	Treatment	Route	Conc (Active)	Batch No.	Dose
1	3	No Treatment				
2	3	Combination Test Formulation (Example 15) Abamectin Oxfendazole Levamisole	Oral	 1.0g/L 22.6g/L 37.5g/L	LB99/03	1mL/5kg
3	4	Combination Test Formulation (Example 15) Abamectin Oxfendazole Levamisole	Pour On	 1.0g/L 22.6g/L 37.5g/L	LB99/03	1mL/2.5kg
4	3	Commercial Formulations Virbamec (abamectin) Systamex (oxfendazole) Young's Levamisole (levamisole)	 Oral Oral Oral	 0.8g/L 90.6g/L 37.5g/L	 33245 V2 153 MV-5-004 3183	Per Label

RESULTS

Table 4

Faecal Egg Counts (strongyle eggs/gram)

Group	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 13
1	Nil (Untreated control)	685	439	811	521	380	311	208	249	249	272
2	Oral Combination Formulation (Example 15)	491	2	0	0	0	0	0	0	0	0
3	Pour-on Combination Formulation (Example 15)	441	10	0	0	0	0	0	0	0	0
4	Oral Commercial Formulations	701	2	0	0	0	0	0	0	0	0

Treatment Day = Day 0

Table 5

Total Worm Count - Individual Animal Data

Group	Treatment	Animal No.	Haemonchus placei	Trichostongylus axei	Ostertagia ostertagi	Cooperla Spp.	Oesophagostomum radiatum
1	Untreated	3100	440	1240	340	1900	29
1	Untreated	3101	340	140	3140	2240	6
1	Untreated	3102	220	0	1120	3340	76
2	Oral Comb	3103	0	0	0	0	0
2	Oral Comb	3104	0	0	0	0	0
2	Oral Comb	3105	0	0	0	0	0
3	Pour-on Comb	3106	0	0	0	0	0
3	Pour-on Comb	3107	0	0	0	0	0
3	Pour-on Comb	3108	0	0	0	0	0
3	Pour-on Comb	1079	0	0	0	0	0
4	Oral Comm	3109	0	0	0	0	0
4	Oral Comm	1077	0	0	0	0	0
4	Oral Comm	1078	0	0	0	0	0

In the drawings,

Figure 1 is a plot of Avermectin B1a blood levels (ng/mL) against time (hours) for the different modes of administration,

Figure 2 is a plot of Levamisole blood levels (μg/ml) against time (hours) for the different modes of administration,

Figure 3 is a plot of Oxfendazole blood levels (μg/ml) against time (hours) for the different modes of administration, and

Figure 4 is a plot of Fenbendazole blood levels (μg/ml) against time (hours) for the different modes of administration.

RESULTS

The trial confirmed that the combination formulation (example 15, Batch LB99/03) was a highly effective anthelmintic in cattle both orally and as a pour-on. This is demonstrated by a complete (100%) reduction in faecal egg count two days following treatment and a greater than 99.9% reduction of gastrointestinal nematodes compared with untreated controls.

The blood profiles of abamectin, levamisole and oxfendazole with the text product (example 15) was given orally were essentially similar to those achieved in animals receiving the three commercial formulations of these actives. The higher blood levels of

oxfendazole in the combination product were probably because of the oily nature of the formulation. Such differences have been seen previously between oily and aqueous benzimidazole formulations (Hennessy 1996). The blood profiles in pour-on treated animals shows that levamisole blood levels were similar to those achieved orally, the abamectin were ten times lower than with the commercial oral abamectin formulation and low levels of oxfendazole and fenbendazole (a metabolite) were detected in blood.

- **Sheep**

Nine 17-20 month old merino hoggets naturally infected with worms were allocated on the basis of parasite faecal egg count (FEC) to 3 treatment groups of 3 animals, each group having a similar mean FEC.

At treatment day (day 0) animals in Group 1 received no treatment (untreated controls), Group 2 received the combination product orally as detailed in example 14 (Batch No. LB99/01) and Group 3 received the same actives using 3 separate commercial formulations (abamectin, levamisole and albendazole) given orally.

Faeces for parasite FEC and blood for blood levels of active were collected at various times following treatment (day 0). Animals were slaughtered at day 14 following treatment, with total numbers of worms counted within the gastrointestinal tract.

The results are presented below and in the drawings.

Table 6

Treatment Groups

Group	No. of Animals	Treatment	Route	Conc (Active)	Batch No.	Dos
1	3	No Treatment (controls)				
2	3	Abamectin Albendazole Levamisole	Oral	1.0g/L 23.8g/L 37.5g/L	LB99/01	1mL/5kg
3	3	Virbamec (abamectin) Valbazen (albendazole) Young's Levamisole (levamisole)	Oral	0.8g/L 19g/L 37.5g/L	33425 V2 7003 3183	Per Label

Results

Table 7

Faecal Egg Counts (Strongyle Eggs/gram)

Group	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 13
1	Nil	2205	2093	2543	1198	1930	3078	1351
2	Oral Combination Formulation (Example 14)	2213	722	0	0	0	0	0
3	Oral Combination Formulations	2475	150	0	0	0	0	0

Table 8

Total Worm Counts - Individual Animal Data

Abomasal Nematodes

Group	Treatment	Animal No.	Haemonchus contortus	Ostertagia circumcincta	Trichostrongylus axel
1	Nil	1	760	720	580
1	Nil	2	80	2220	840
1	Nil	3	180	1520	560
2	Oral Comb	4	0	0	0
2	Oral Comb	5	0	0	0
2	Oral Comb	6	0	0	0
3	Oral Comm	7	0	0	0
3	Oral Comm	8	0	0	0
3	Oral Comm	9	0	0	0

Table 9

Total Worm Counts - Individual Animal Data

Small and Large Intestine Nematodes

Group	Treatment	Animal No.	Cooperia Spp.	Nematodirus spathiger	Trichostrongylus colubriformis	Oesophagostomum columbianum
1	Nil	1	340	1480	8260	144
1	Nil	2	760	1760	6040	137
1	Nil	3	60	1540	4800	134
2	Oral Comb	4	0	0	0	0
2	Oral Comb	5	0	0	0	0
2	Oral Comb	6	0	0	0	0

3	Oral Comm	7	0	0	0	0
3	Oral Comm	8	0	0	0	0
3	Oral Comm	9	0	0	0	0

In the drawings

Figure 5 is a plot of Avermectin B1a blood level (ng/mL) against time (hours) for the oral combination against a commercial oral formulation,

Figure 6 is a plot of Levamisole blood level (µg/ml) against time (hours) for the oral combination against a commercial oral formulation, and

Figure 7 is a plot of Albendazole blood levels (µg/ml) against time (hours) for the oral combination against a commercial oral formulation.

Results

The trial confirmed that the combination formulation (example 14, Batch No. LB99/01) was a highly effective anthelmintic when given orally in sheep. This was demonstrated by a complete (100%) reduction in faecal egg count by day 2 and a greater than 99.9% reduction in all major worm species in the gastrointestinal tract.

The blood profiles of abamectin, levamisole and albendazole with the test product (example 14) were essentially similar to those achieved in animals receiving commercial formulations of the same three actives.

DATED THIS 4th DAY OF July 1999
A.J. PARK & SON
PER
AGENTS FOR THE APPLICANT